

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Weiss <i>et al.</i>
Serial No.	:	08/486,313
Filed	:	June 7, 1995
For	:	MULTIPOTENT NEURAL STEM CELL COMPOSITIONS
Examiner	:	A-M. Baker
Group Art Unit	:	1632

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

I, E. EDWARD BAETGE, hereby declare and state as follows:

1. I received my B.S., and Ph.D. degrees from the University of San Diego & Cornell University in 1978 and 1983, respectively. I am a named inventor. I have been working in the field of cell and molecular neurobiology since 1978, and working with neural stem cells since 1991.
2. I am currently employed as Chief Scientific Officer at Modex Therapeutiques SA (Lausanne, Switzerland) in the field of cell and molecular Biology. Prior to this, I worked at CytoTherapeutics, Inc. (now StemCells, Inc.), the company that is the exclusive licensee of this patent application. At CytoTherapeutics, I was in charge of all scientific research. I am a named inventor on this patent application and am intimately familiar with the neural stem cell cultures and methods of using them that this application describes (such as the pending claims which are directed to methods for transplanting neural stem progeny into a host).
3. I understand that the Examiner has rejected the pending claims under 35 U.S.C. §112 contending that “[t]he claims are not enabled because the transplantation of multipotent neural stem cell progeny into a host has not been demonstrated to provide any therapeutic benefit to the host.”
4. I make this declaration to rebut the Examiner's rejection, with which I do not agree. In view of the express statements in the specification regarding transplantation of neural stem cell cultures and the voluminous experimental evidence that has been accumulated, in my opinion, the ordinarily skilled artisan would be able to routinely transplant the described neural stem cell cultures without

undue experimentation at the filing date of this application. I am also of the opinion that although the claimed methods do not state or require any therapeutic benefit, that transplantation of neural stems into a host has been clearly demonstrated to confer a therapeutic benefit, and that the ordinarily skilled artisan would believe that such transplantation would provide a therapeutic benefit to the host.

5. Prior to this invention, the operating dogma in neurobiology was that the brain was relatively quiescent, and that there was no "stem cell" that could be proliferated and then differentiated to form the three major cell types in the central nervous system (i.e., neurons, astrocytes and oligodendrocytes). The neural stem cells described for the first time in this invention, and the ability for the art (provided by the inventors here) to obtain proliferating cultures of those cells, has been widely hailed as a landmark in neurobiology. In fact, over 500 published references cite one of the early publications that describe this invention (Reynolds and Weiss, *Science* 255:1707-10 (1992)). A number of these publications demonstrate that a therapeutic benefit is conferred when neural stem cells are transplanted into a host. I will discuss these in detail below.

6. As a general matter, it is my view, based on my knowledge of the field and based on the voluminous citations to the inventors' work, that researchers of ordinary skill in the relevant arts clearly recognized the importance of the Applicants' invention and relied upon it in their subsequent work. In my opinion, the Applicants' invention discloses paradigm-shifting technology and is a vitally important finding in the field of neurobiology. I am of the firm view that the claimed methods for transplanting neural stem cells are enabled, and provide my detailed thoughts below.

7. I believe that the neural stem cell cultures of this invention are particularly suited for transplantation, since, until this invention, cultures of proliferating neural cells have not been available to the art. Further, in the claimed transplantation methods, the tissue source is well-defined, reproducible, and is not derived from an oncogene-immortalized cell line (thus being non-tumorigenic). See, specification, pg. 11, lines 15-20. In fact, I concluded some years ago that such neural stem cell cultures are "ideal" for nervous system transplantation. See, *Baetge et al.*, 695 *Ann N.Y. Acad. Sci.*, pp. 285-291 (1993). Multiple other workers in the field have reached the same conclusion.

8. I understand that the specification contains explicit guidance about how to transplant CNS neural stem cells (see, specification, pg. 36, line 10, to pg. 42, line 13; pg. 68, line 16, to pg. 69, line 18; pg. 78, line 17, to pg. 71, line 6; pg. 96, line 12, to pg. 97, line 28), and that the specification provides working examples of neural stem cell transplantation in various disease models, including, e.g., Huntington's disease, Parkinson's disease, and cardiac arrest. See, e.g., specification, pp. 96-101. In addition, the specification teaches and discloses the types of diseases to which the invention is directed. (See pg. 40, lines 9-18). The specification also provides exemplary teaching of where to transplant the cells of the claimed invention. (See, e.g., pg. 38, lines 17-30). Further, the specification teaches and discloses how to monitor the transplanted cells. (See pg. 39, lines 16-31). Additionally, the specification teaches and discloses how to get the transplanted cells to proliferate *in vivo*. (See pg. 52, line 14 through page 47, line 26). In addition, I believe that the ordinarily skilled artisan would know how to actually carry out the step of transplanting the neural stem cell cultures of this invention according to the claimed methods given that the art is replete with examples of transplantation of various other tissues or cells into various parts of the brain (witness, e.g., transplantation of porcine neural cells and fetal human cells). For this reason, in my view it cannot be disputed that the ordinarily skilled artisan, with the specification in hand, would be able to transplant neural stem cell cultures into a host; that is, the ordinarily skilled artisan would know how to use the invention as claimed. I do not believe that the Examiner disputes this; rather I believe that the Examiner is asking for additional proof that the ordinarily skilled artisan would believe that making such a transplantation would confer a therapeutic benefit. I have provided that proof below.

9. I understand that the specification provides forty-five (45) *in vitro* and *in vivo* examples relating to generation and use of neural stem cell cultures. Among these examples are detailed various standard, well-accepted animal models of various human diseases, including, e.g., animal models for Parkinson's disease and Huntington's disease. Applicants also disclosed treatment of neurodegenerative disease using progeny of human neural stem cells proliferated *in vitro*; remyelination in myelin deficient rats using neural stem cell progeny proliferated *in vitro*; remyelination in human neuromyelitis optica; and remyelination human Pelizaeus-Merzbacher disease. (See Specification, Examples 14-17).

10. I have read Dr. Hammang's December 2000 Declaration and I agree with his views expressed therein. In particular, Dr. Hammang gave two reasons why the ordinarily skilled artisan

would reasonably expect that the claimed transplantation methods would provide a therapeutic benefit; (1) that the transplanted neural stem cell cultures secrete cellular products which are capable of providing a therapeutic benefit to the host, and (2) that the neural stem cell cultures exhibit tissue-specific differentiation upon transplantation. I agree with Dr. Hammang. In my view, either of these facts would inescapably lead the ordinarily skilled artisan to conclude that transplantation of such neural stem cell cultures would have a reasonable expectation of success in providing a therapeutic benefit to the host.

A. Transplantation of Neural Stem Cell Progeny According to the Claimed Methods For Delivery of Cellular Products Provides a Therapeutic Benefit

11. Applicants' neural stem cell cultures have been shown to be a useful tool for delivery of secreted cellular products which provide a therapeutic benefit when transplanted into the host. I draw the Examiner's attention to three publications that demonstrate that transplantation of cultures of neural stem cells that have been genetically modified to secrete nerve growth factor ("NGF"), according to the claimed methods, provides a therapeutic benefit.
 12. Andsberg et al., "Amelioration Of Ischaemia-Induced Neuronal Death In The Rat Striatum By NGF-Secreting Neural Stem Cells", *Europ. J. Neuroscience*, 10, pp. 2026-2036 (1998) (copy attached as Ex. 1) reports that transplantation of NGF-secreting neural stem cell cultures into the adult rat striatum following middle cerebral artery occlusion ameliorated the death of striatal projection neurons that would have otherwise died due to the ischaemic insult. This clearly demonstrates a therapeutic benefit of the claimed methods.
 13. Carpenter et al., "Generation and Transplantation of EGF-responsive Neural Stem Cells Derived From GFAP-hNGF Transgenic Mice", *Exp. Neurology*, 148, pp. 187-204 (1997) (copy attached as Ex. 2) reports that transplantation of NGF-secreting neural stem cell cultures into the adult rat striatum produced dense sprouting of p75 neurotrophin receptor-positive fibers emanating from the underlying basal forebrain – a significant morphological change compared to controls, which I believe would be considered a therapeutic benefit in hosts where such neuronal regeneration and sprouting were desired. The authors conclude that "[t]he use of neural stem cells for transplantation into the CNS offers a number of advantages over transplantation of primary tissue or other cell lines." See p. 202. This to me also clearly demonstrates a therapeutic benefit of the claimed methods.

14. Kordower et al., "Grafts of EGF-responsive Neural Stem Cells Derived From GFAP-hNGF Transgenic Mice: Trophic and Tropic Effects in a Rodent Model of Huntington's Disease", *J. Comp. Neurol.*, 387, pp. 96-113 (1997) (copy attached as Ex. 3) reports that intrastratal transplantation of NGF-secreting neural stem cell cultures into an adult rat model of Huntington's disease (the well accepted and widely used quinolinic acid lesion model) resulted in sparing of striatal neurons immunoreactive for glutamic acid decarboxylase, choline acetyltransferase, and neurons histochemically positive for nicotinamide adenosine diphosphate. In addition the NGF-secreting transplants produced robust sprouting of cholinergic fibers from subjacent basal forebrain neurons. The authors conclude that "[t]hese data indicate that cellular delivery of hNGF by genetic modification of stem cells can prevent the degeneration of vulnerable striatal neural populations, including those destined to die in a rodent model of HD and supports the emerging concept that this technology may be a valuable therapeutic strategy for patients suffering from this disease." See p. 96. In my view, the authors clearly demonstrated a therapeutic benefit and clearly expressed their view that the results in the model are predictive of the human condition. For this reason, these data also clearly demonstrates a therapeutic benefit of the claimed methods.

B. Transplantation of Neural Stem Cell Progeny According to the Claimed Methods For Tissue-Specific Differentiation Provides a Therapeutic Benefit

15. Applicants' neural stem cell cultures have been shown to be useful tool for tissue-specific differentiation to provide a therapeutic benefit when transplanted into the host. I draw the Examiner's attention to several new publications that demonstrate that transplantation of cultures of neural stem cells provide such a therapeutic benefit.

16. Qu et al., "Human Neural Stem Cells Improve Cognitive Function of Aged Brain", *Ageing*, 12, pp. 1127-1132 (2001) (copy attached as Ex. 4) report that when human neural stem cells were transplanted into aged rats (about 24 months old), according to the claimed methods, the cells not only survived, but also retained their multipotency and migratory ability. The results show that the human neural stem cells not only successfully differentiated into neurons and astrocytes, but importantly "both neurons and astrocytes migrated into the cortex and hippocampus in a well-defined and organized pattern in the brain." See p. 1132. Finally, the results demonstrate

significantly improved cognitive function (in the standard and well accepted Morris water maze model). In my view this clearly demonstrates a therapeutic benefit of the claimed methods.

17. Akiyama et al., "Transplantation of Clonal Neural Precursor Cell Derived From Adult Human Brain Establishes Functional Peripheral Myelin in the Rat Spinal Cord", *Exp. Neurol.*, 167, pp. 27-39 (2001) (copy attached as Ex. 5) reports that human neurosphere cultures (*i.e.*, an expressly disclosed embodiment of the neural stem cell cultures of this invention) when transplanted (according to the claimed methods) into a demyelinated adult rat spinal cord produced extensive remyelination with a peripheral pattern similar to Schwann cell myelination characterized by large cytoplasmic and nuclear regions, a basement membrane, and P0 immunoreactivity. Importantly, "the remyelinated axons conducted impulses at near normal conduction velocities". *See* p. 27. In my view this clearly demonstrates a therapeutic benefit of the claimed methods.

18. Kurimoto et al., "Transplantation of Adult Rat Hippocampus-Derived Neural Stem Cells into Retina Injured By Transient Ischemia", *Neuroscience Letters*, 306, pp. 57-60 (2001) (copy attached as Ex. 6) reports transplantation of rat neural stem cell cultures into the eyes of adult rats that underwent ischemia-reperfusion injury. The *in vivo* retinal ischemia-reperfusion model is a standard (and well accepted) experimental model that has been used to investigate the damage to the retina induced by transient ischemia. The authors report that in the eyes with the ischemia insult, the intravitreally injected neural stem cells invaded the retinal ganglion cell layer within a week of the transplantation and were identified in the retinal inner nuclear layer two weeks after the transplantation. At four weeks the donor cells were integrated into the host retina and expressed Map2ab, which indicated that the cells had differentiated into mature neurons. By comparison, in the control, none of the transplanted cells migrated to the retina. The authors conclude that "neuronal stem cells are good candidates to reconstruct the neural circuitry of ischemic injured retina, and show the potentiality of therapeutic transplantation using neuronal stem cells on retinal impairments that are generally regarded as incurable." *See* p. 59. I conclude from this that, both the authors (and myself) believe that this clearly demonstrates a therapeutic benefit of the claimed methods.

19. Likewise, Nishida et al., "Incorporation and Differentiation of Hippocampus-Derived Neural Stem Cells Transplanted in Injured Adult Rat Retina", *Investigative Ophthalmology & Visual Science*, 41, pp. 4268-4274 (Dec 2000) (copy attached as Ex. 7) report that transplantation of neural

stem cells into mechanically injured adult retina results in incorporation and subsequent differentiation of the grafted stem cells into neuronal and glial lineages. Importantly, the authors conclude that “[n]eural stem cells are expected to be useful clinically for replacing damaged neurons or for ex vivo gene therapy.” See, p. 4271. This statement confirms my statements throughout this declaration, and I believe is reflective of the general opinion of those of ordinary skill in the art.

20. I also refer the Examiner to Milward *et al.*, 50 J. Neurosci. Res. 862-871 (1997) (“Milward”) (Ex. 8). *Milward* successfully transplanted canine CNS neural stem cells both into rat and into a shaking (*sh*) pup myelin mutant dog (a model of human myelin diseases). In *Milward*, canine neural stem cell cultures were transplanted into the myelin-deficient (*md*) rat spinal cord, resulting in the production of myelin by graft-derived cells (see, *Milward*, pg. 868, col. 1, 2nd para.), demonstrating that transplanted CNS neural stem cells can differentiate in the recipient to form myelin-producing oligodendrocytes and therapeutically provide myelin to recipients. The *Milward* shaking pup canine model is a particularly harsh model of dys-myelination. The pups are born with little to no myelin sheath. The *Milward* data demonstrate that that the present invention can be used to provide myelination in an almost myelin-free *in vivo* environment. In addition, as *Milward* reports, the grafted cells had integrated normally into the adult *sh* pup cytoarchitecture. See, p. 867, right column. This is powerful support that transplantation of such neural stem cell cultures would be useful in providing a therapeutic benefit to the host. This is particularly true in “real-life” disease situations where de-myelination is a relatively slow process occurring not globally but in patches.

The Akiyama paper discussed above confirms that the remyelination effected by transplantation of neural stem cells (as demonstrated in *Milward*), in fact, is functional and therapeutic (as it restores near normal conduction velocities to the remyelinated axons).

I understand that the Examiner has indicated that “the formation of myelin, as described by Milward *et al.*, did not result in producing a therapeutic effect in the animal. Thus, Milward *et al.* does not demonstrate application of the claimed method to produce a therapeutic effect.” (Office Action, pages 4-5). I disagree with the Examiner’s characterization of *Milward*, and, it is my opinion that the Akiyama paper discussed herein confirms my earlier conclusions regarding *Milward*. In my view, the ordinarily skilled artisan would expect that replacement of myelin on demyelinated axons would confer a therapeutic benefit, since the “replaced” myelin provides the missing insulation that is necessary to support impulse conduction. The Examiner does not explain

why she believes that *Milward* does not produce a therapeutic effect, and I believe that Akiyama directly supports that a therapeutic benefit is conferred.

21. I also refer the Examiner to *Zhang et al.*, 96 Proc. Natl. Acad. Sci. USA 4089-94 (1999) ("*Zhang*") (Ex. 9). *Zhang* reports similar results, in which neural stem cell cultures were generated from both juvenile and adult rats and used to produce myelin-forming cells, and when transplanted into *md* rats, those cells produced "robust myelination". See, *Zhang*, pp. 4093-94. For the reasons discussed above, this too, in my view, would lead the ordinarily skilled artisan to conclude that transplantation of such neural stem cell cultures would be useful in providing a therapeutic benefit to the host.

I understand that the Examiner has stated that "this 'robust myelination' in fact did not produce a therapeutic effect in the host." (Office Action, page 5) However, the Examiner has not provided any evidence for this assertion. Moreover, I disagree with the Examiner's contention. As noted above, the ordinarily skilled artisan would expect that replacement of myelin to demyelinated axons would confer a therapeutic benefit. I believe that Akiyama directly supports that a therapeutic benefit is conferred.

22. Likewise, I also refer the Examiner to *Brüstle et al.*, 16 Nature Biotechnol., pp. 1040-1044 (1998) ("*Brüstle*") (Ex. 10). *Brüstle* describes the implantation of fetal human CNS progenitor cells into mice that "acquire an oligodendroglial phenotype and participate in the myelination of host axons".

The Examiner has stated that, because *Brüstle*'s experiments were conducted in healthy animals, no therapeutic effect was demonstrated because the "function of the cells upon transplantation is not sufficient to support enablement because there is not sufficient guidance for using the transplantation method therapeutically in diseased animals." (Office Action, page 5).

I disagree. *Brüstle* did demonstrate that the transplanted cells functioned as expected after transplantation -- and participated in the myelination of host axons. I believe that demonstration of ability to myelinate axons (as *Brüstle*'s experiments showed) would lead one of ordinary skill in this art to reasonably expect that these cells would provide a therapeutic benefit in the appropriate disease environment. Additionally, in view of Akiyama, I do not believe the Examiner can maintain the position that the claimed methods do not produce a therapeutic benefit in demyelinating/demyelinating disorders.

23. Others have also demonstrated that these neural stem cell cultures, when transplanted, are, in fact, capable of replacing the critical functions of lost or deficient neural populations. See, e.g., Flax *et al.*, 16 Nature Biotechnol., pp. 1033-1039 (1998) ("Flax") (Ex. 11). Flax showed that transplantation of CNS neural stem cells provides a therapeutic benefit in the meander tail (*mea*) mouse, a well-known and well accepted mouse mutant model characterized by a cell-autonomous failure of granule neurons to develop or survive in the cerebellum, especially the anterior lobe. Flax transplanted human CNS neural stem cells into newborn *mea* cerebella and confirmed that the human neural stem cells provided "replacement neurons" with the "definitive size, morphology, and location of cerebellar granule neurons (Fig. 6E-G)" (Flax, pg. 1037, col. 2, 2nd para).

Thus, in my view, Flax showed that transplanted CNS neural stem cells are able to differentiate in the recipient to form granule neurons and to therapeutically provide replacement neurons to recipients (such as these *mea* mice). In my view, this ability to replace damaged or missing neurons is unequivocal evidence of a therapeutic benefit upon transplantation of neural stem cell cultures, as claimed here. Moreover, my view has also been supported independently by other workers in the field who reviewed this Flax, Nature Biotech paper. See Zigova & Sanberg, 16 Nature Biotechnol., pp. 1007-1008 (1998) ("Zigova") (Ex. 12) which states that the Flax data "provides strong evidence that the NSCs [neural stem cells] are able to perform *in vitro* and *in vivo* all the critical functions previously described for their rodent counterparts" (Zigova, pg. 1007, middle column).

I understand that the Examiner has indicated that the "replacement neurons" in fact did not produce a therapeutic effect. Thus, it is unclear how the claimed method can be used for therapy, since no teachings are provided to allow the skilled artisan to produce "replacement neurons" for therapy." (Office Action, page 6). I disagree with this position. Flax transplanted NSCs into a murine model characterized by a failure of granule neurons to develop or survive in the cerebellum. The results of these experiments with this model demonstrate that the NSCs differentiated into neurons of the appropriate size, morphology, and location. Flax concluded that "engrafted NSCs of human origin appear sufficiently plastic to respond appropriately to varying local cues for lineage determination."

Thus, in this animal model that is lacking a certain type of neuron, Flax demonstrated that transplantation of neural stem cell cultures replaced the missing neurons. In my opinion, these results are clear evidence of the therapeutic effect of the claimed invention because the results demonstrate that transplantation of neural stem cells according to the claimed methods results in tissue-specific differentiation.

24. I also referred to Fricker *et al.*, 19 J. Neurosci., pp. 5990-6005 (1999) ("Fricker") (Ex. 13). Fricker showed that when CNS neural stem cells were transplanted into neurogenic regions in the adult rat brain, the subventricular zone, and hippocampus, the *in vitro* propagated cells migrated specifically along the routes normally taken by endogenous neuronal precursors: along the rostral migratory stream to the olfactory bulb and within the subgranular zone in the dentate gyrus. The *in vitro* propagated cells exhibited site-specific neuronal differentiation in the granular and periglomerular layers of the bulb and in the dentate granular cell layer. Additionally, the CNS neural stem cells also exhibited substantial migration within the non-neurogenic region, the striatum, and showed differentiation into both neuronal and glial phenotypes. Thus, in my view, Fricker confirmed the ability of the human neural stem cells to respond *in vivo* to guidance cues and signals that can direct their differentiation along multiple phenotypic pathways.

The Examiner noted that the ability of human NSCs to respond *in vivo* to guidance cues and signals that can direct their differentiation "has not as yet been exploited to develop methodology to produce a therapeutic effect." (Office Action, page 6). Again, I do not agree with this characterization. In my view, one skilled in the art would recognize the importance of Fricker's discovery that the NSC cultures are able to respond *in vivo* to surrounding signals and cues in the treatment of a variety of CNS diseases and disorders. Thus, it is my opinion that the work reported by Fricker could be used by those skilled in the art to achieve a therapeutic benefit in a patient being treated.

25. I also draw the Examiner's attention to Aboody et al., 97 Proc. Natl. Acad. Sci. USA, pp. 12846-51 (November 2000) ("Aboody") (Ex. 14). Aboody expressly states (see, e.g., throughout the paper, and particularly Abstract and p. 12851) that neural stem cell cultures provide a transplantation "platform" since upon transplantation those cells can both continue to express a foreign gene and migrate in a site specific fashion in host tissue for "dissemination of therapeutic genes".

In response, the Examiner has states that "Aboody et al., does not teach the steps required to transplant NSCs in a manner such that a therapeutic effect is produced." (Office Action, pages 6-7). To the contrary, it is my belief that Aboody's characterization of the neural stem cell cultures as a transplantation "platform" is sufficient evidence of a therapeutic benefit -- specifically, those skilled in the art would be able to use these cells in the claimed methods to achieve such a therapeutic benefit without undue experimentation.

26. Finally, I draw the Examiner's attention to several additional publications that, in my view, clearly demonstrate that the art is of the view that the claimed transplantation methods would provide a therapeutic benefit. In particular, see e.g. Ourednik et al., Novartis Foundation Symposium 231, Pub. John Wiley & Sons, Ltd. (2000) (Ex. 15), which is titled "Neural Stem Cells Are Uniquely Suited For Cell Replacement and Gene Therapy in the CNS". This title alone captures the sentiments expressed throughout my declaration. See also Vescovi et al., "Isolation and Intracerebral Grafting of Nontransformed Multipotential Embryonic Human CNS Stem

Cells", J. Neurotrauma, 16, pp. 689-693, p. 689 (1999) (Ex. 17), which states "the use of human embryonic CNS stem cells should provide a reliable solution to some of the major problems that pertain to this field ...".

27. For all the foregoing reasons, I believe that the Examiner should withdraw the rejection and allow the pending claims.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.



E. Edward Baetge

Signed at Lausanne, Switzerland
this 30 day of July, 2001

TRA 1541674v1